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Published in:
Journal of Veterinary Internal Medicine

DOI:
[10.1111/jvim.15387](https://doi.org/10.1111/jvim.15387)

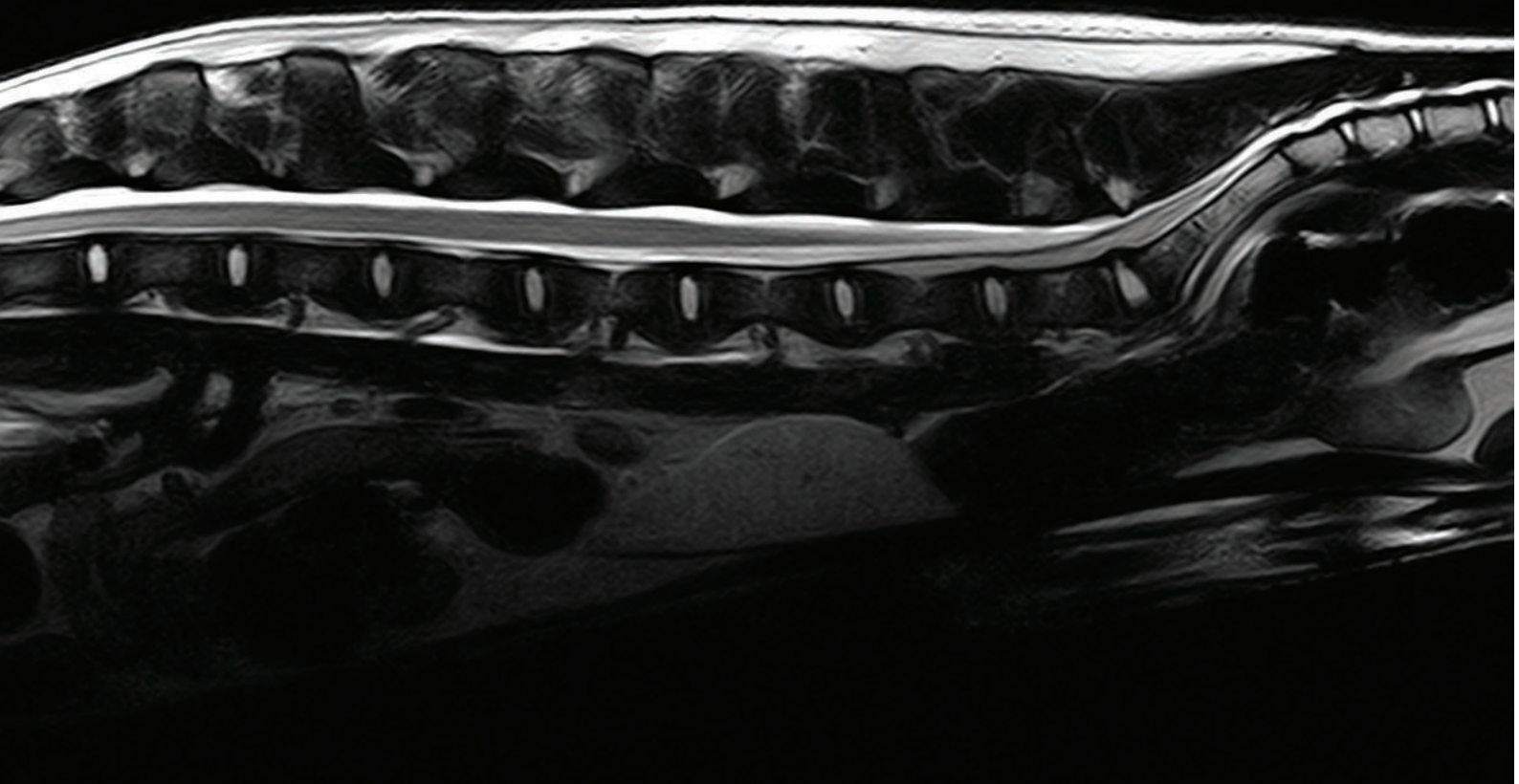
Publication date:
2019

Document version
Publisher's PDF, also known as Version of record

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Citation for published version (APA):
Relun, A., Cesbron, N., Bourdeau, P., Dorso, L., Brement, T., Assié, S., Christensen, H., & Guatteo, R. (2019). Atypical actinobacillosis affecting hind limbs and lungs in a single beef cattle herd. *Journal of Veterinary Internal Medicine*, 33(1), 297-301. <https://doi.org/10.1111/jvim.15387>

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CASE REPORT

Atypical actinobacillosis affecting hind limbs and lungs in a single beef cattle herd

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Actinobacillosis usually is a sporadic infection that affects the tongue in cattle ("wooden tongue") with possible spread to the digestive tract. Two 4-year-old Rouge-des-Prés cows from a single French beef herd were referred for chronic (2–6 months) swelling and cutaneous nodules in the distal hind limbs. In addition to cutaneous signs, physical examination disclosed cachexia, lameness, lymphadenitis of the hind limbs, and pneumonia in both cows. Cytologic examination of direct skin smears was inconclusive, and no parasites were observed in examination of multiple skin scrapings. Histopathological examination of skin and lung biopsy specimens identified chronic, diffuse, severe pyogranulomatous dermatitis, associated with Splendore-Hoeppli phenomenon and intralesional Gram-negative bacteria. Cultures from skin, lymph nodes, and lungs (both cows were euthanized for welfare reasons) identified a *Pasteurellaceae* organism, confirmed as *Actinobacillus lignieresii* by partial sequencing of the *rpoB* gene. This report emphasizes that actinobacillosis can appear as a small outbreak in cattle with cutaneous and respiratory signs.

KEYWORDS

Actinobacillus lignieresii, cattle, dermatitis, pneumonia

1 | HISTORY AND CLINICAL FINDINGS

In spring 2015, two 4-year-old Rouge-des-Prés cows from a single 100-cow commercial beef farm were referred to the Clinic for Ruminants at Oniris, France, for investigation of chronic and severe swelling and cutaneous nodules in the hind limbs. At the onset of the disease, the cows were housed in a free-stall barn bedded with straw and fed a total mixed ration based on corn and grass silage. The herd had a history of paratuberculosis and chorioretinitis. The farmer first observed slight lameness and swelling in a hind limb on both cows 6 months (cow A) and 2 months (cow B) before admission. A few weeks after the onset of the swelling, he noticed cutaneous nodular lesions on the affected limbs. At this stage, the cows were treated with long-acting oxytetracycline (20 mg/kg IM q72h, 6 days) and tolafenamic acid (2 mg/kg IM q48h, 4 days) but failed to improve and gradually lost weight. A third cow in the same herd also

developed cutaneous nodules, with lesions limited to the udder skin. This cow was not referred to our clinic.

On presentation, both cows had poor body condition (body condition score of 1 and 2 out of 5 for cows A and B, respectively) despite maintaining normal appetite, moderate tachypnea (40/min and 60/min for cow A and cow B, respectively) with wheezes audible bilaterally in the cranioventral area on lung auscultation, and a locomotion score of 2 (mildly lame) according to a previously published scale.¹ Detailed clinical examination of the limbs and udder disclosed extensive firm swelling extending from the coronary band to the stifle in the left hind limb (cow A) or both hind limbs (cow B), with multiple single to coalescent cutaneous and SC nodules of various sizes (from 0.5 to 10 cm in diameter) on the affected hind limbs as well as on the udder skin of cow A (Figure 1). These nodules were freely movable, slightly compressible to firm on palpation, and some of them were ulcerated with crusts and bled easily. They were associated with numerous purulent fistulae. Cow A also had mastitis, with the 4 quarters being firm, without pain on palpation, secreting creamy caseous pale yellow purulent material, associated with enlargement of the

Abbreviation: PCR, polymerase chain reaction.

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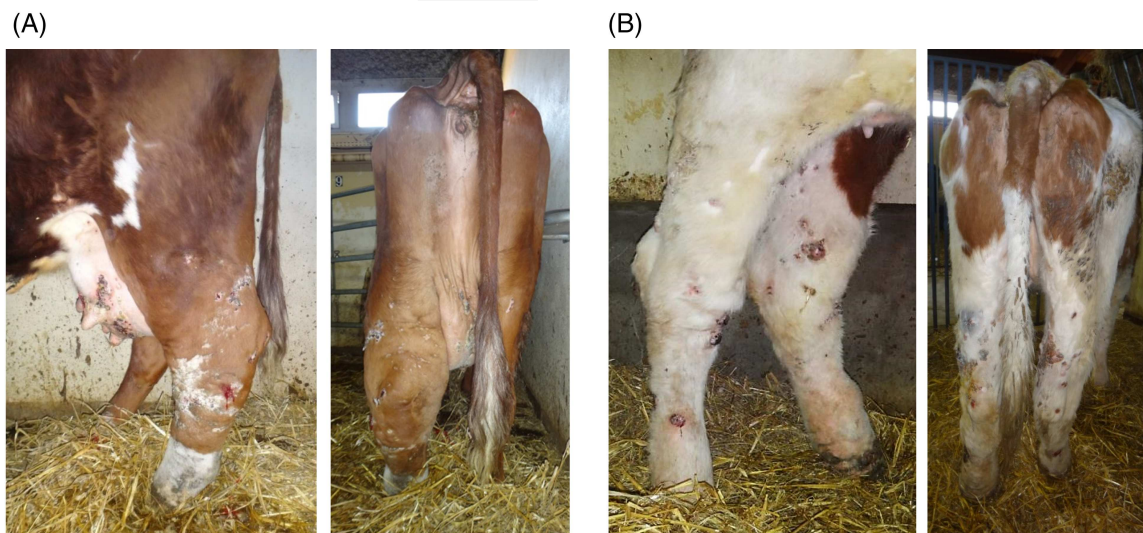


FIGURE 1 Cutaneous and SC nodules in the hind leg and the udder associated with swelling of the affected leg in two Rouge-des-Prés cows with cutaneous actinobacillosis. A, Cow A. B, Cow B

regional lymph nodes. Cow B had mild diarrhea with homogenous pasty green feces with little odor.

At this time, given the clinical findings, the differential diagnosis for cutaneous signs included primarily a cutaneous infection caused by an actinomycetes organism (*Actinobacillus* sp., *Nocardia* sp., *Actinomyces* sp.), a cutaneous infection caused by other bacteria secondary to primary leg mange or fungal infection, and cutaneous tuberculosis. Regarding the respiratory signs, the cows were assumed to have either thromboembolic pneumonia resulting from spread of organisms from skin lesions or primary infectious bronchopneumonia. In cow A, mastitis was considered to be caused by the spread of organisms found in the skin lesions or to a primary infection by bacteria causing pyogranulomatous mastitis (*Mycoplasma* sp., *Nocardia asteroides*, *Arcanobacterium pyogenes*, *Cryptococcus neoformans*, *Prototheca zopfii*). The differential diagnosis for diarrhea with severe weight loss and normal appetite in cow B included primarily clinical paratuberculosis or chronic gastrointestinal parasitism by a trematode or nematode.

2 | ANCILLARY TESTS

Smears prepared using a fast-acting variation of May-Grünwald Giemsa stain (RAL 555 kit, RAL Diagnostics, France) made from direct impression or pus from skin lesions were inconclusive, and no parasites were observed in multiple skin scrapings. Fungal cultures were negative. Diagnostic tests for *Nocardia* sp. (polymerase chain reaction, PCR), *Mycobacterium* sp. (Ziehl-Neelsen stain), and capripox-virus (PCR) were negative on skin biopsy specimens. Histopathological examination of the skin biopsy specimens identified chronic, diffuse, severe pyogranulomatous dermatitis, associated with Splendore-Hoeppli phenomenon and Gram-negative rod-shaped organisms at their center (hematoxylin and eosin stain, Figure 2). This histopathological feature suggested infection by *Actinobacillus lignieresii*.^{2,3} Bacteriological cultures of nodule secretions and skin biopsy specimens identified a small Gram-negative coccobacillus from the *Pasteurellaceae* family.

Antibiotic susceptibility was performed by the disc diffusion method. The isolate was susceptible to amoxicillin, amoxicillin and clavulanic acid, cephalexin, ceftiofur, cefquinome, neomycin, gentamicin, clindamycin, colistin and polymixin B, trimethoprim and sulfamethoxazole, enrofloxacin, marbofloxacin, chloramphenicol, tetracycline, and doxycycline. It exhibited intermediate susceptibility to penicillin G, streptomycin, and flumequine and was resistant to amikacin and erythromycin.

The cows were tested for *Mycobacterium avium* subspecies *paratuberculosis* by Ziehl-Neelsen stain on a fecal smear and serology (indirect ELISA assay). Cow B was positive on both tests, whereas cow A was negative on both.

The chances of clinical recovery were determined to be very poor because of the severe and chronic swelling of the hind limbs and severe mastitis in cow A, clinical paratuberculosis in cow B, and the failure of previous treatment. No treatment was attempted, both

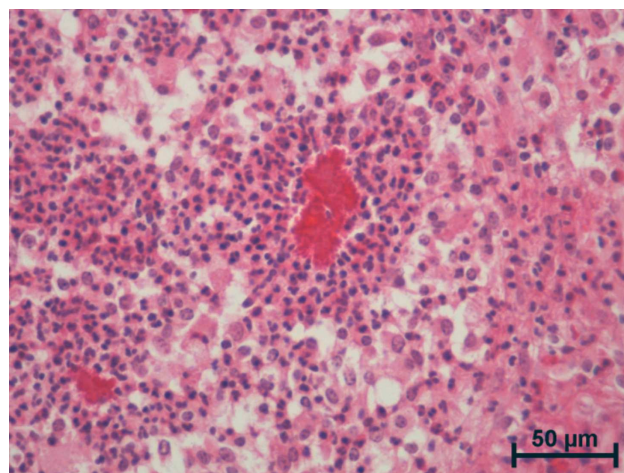


FIGURE 2 Photomicrographs of a lung lesion showing distinctive eosinophilic club-like rosettes surrounded by reactive neutrophils and macrophages (Splendore-Hoeppli phenomenon). Hematoxylin and eosin, $\times 400$

animals were humanely euthanized, and complete necropsy was performed with the farmer's consent.

Relevant findings at necropsy included chronic nodular dermatitis on 1 or both hind limbs associated with SC edema and lymphangitis in the affected limb, and severe weight loss in both cows. In addition, cow A had chronic suppurative thromboembolic pneumonia with multifocal abscesses associated with vessels, affecting cranial and medial lobes as well as cranial parts of the caudal lobes, associated with lymphadenitis of the tracheobronchial lymph nodes, chronic suppurative mastitis, chronic suppurative lymphadenitis in the left precrural, the left and right retromammary and iliac lymph nodes, as well as chronic interstitial nephritis (Figure 3). Cow B also had chronic bronchopneumonia and chronic granulomatous enteritis, typical of *Mycobacterium avium* subspecies *paratuberculosis* infection.

Specimens of lung, udder, and lymph node lesions observed at necropsy were collected for bacterial culture and histopathology. Splendore-Hoeppli phenomenon was observed, associated with pyogranulomatous mastitis, lymphadenitis, and suppurative bronchopneumonia in cow A (Figure 2). Bacterial cultures of the retromammary lymph nodes and lungs yielded a small Gram-negative coccobacillus from the *Pasteurellaceae* family, based on phenotypic characteristics.

Given the clinical, histopathological, and bacteriological findings, atypical actinobacillosis was the primary differential diagnosis. Specimens of mammary lymph nodes from cow A and purulent material from a cutaneous nodule from cow B were sent to the Department of

Veterinary Microbiology (Copenhagen, Denmark) for genetic identification. Polymerase chain reaction was performed to identify the 2 isolates. Primers designed to amplify the *rpoB* gene were used as described previously.⁴ The PCR products were sequenced with the same primers as those used for PCR amplification by Macrogen (Seoul, South Korea). The DNA sequences were assembled by CLC Main Workbench (Qiagen, Aarhus, Denmark), and pairwise comparisons were performed with the EMBOSS water program.⁵ The organisms were confirmed as *A. lignieresii* in both tissues tested with 99.4% DNA similarity to the type strain of the species (strain NCTC 4189^T, GenBank accession number AY170215, <https://www.ncbi.nlm.nih.gov/genbank/>). The 2 strains sequenced using purulent material from a cutaneous nodule and mammary lymph node from the cows in the herd showed 99.4% DNA similarity, indicating that the infection in the herd was not related to a single clone (strain MH426972^T and MH426973^T, GenBank accession number BankIt2120127 and BankIt2120132, respectively).

3 | FOLLOW-UP

Given that at least 3 animals in the herd had been affected, the referring veterinarian was advised to look for any risk factors for wounds of the cattle (eg, sharp objects, dogs biting the cattle) that could have caused wounds in the hind limbs and udders of the cattle, as well as

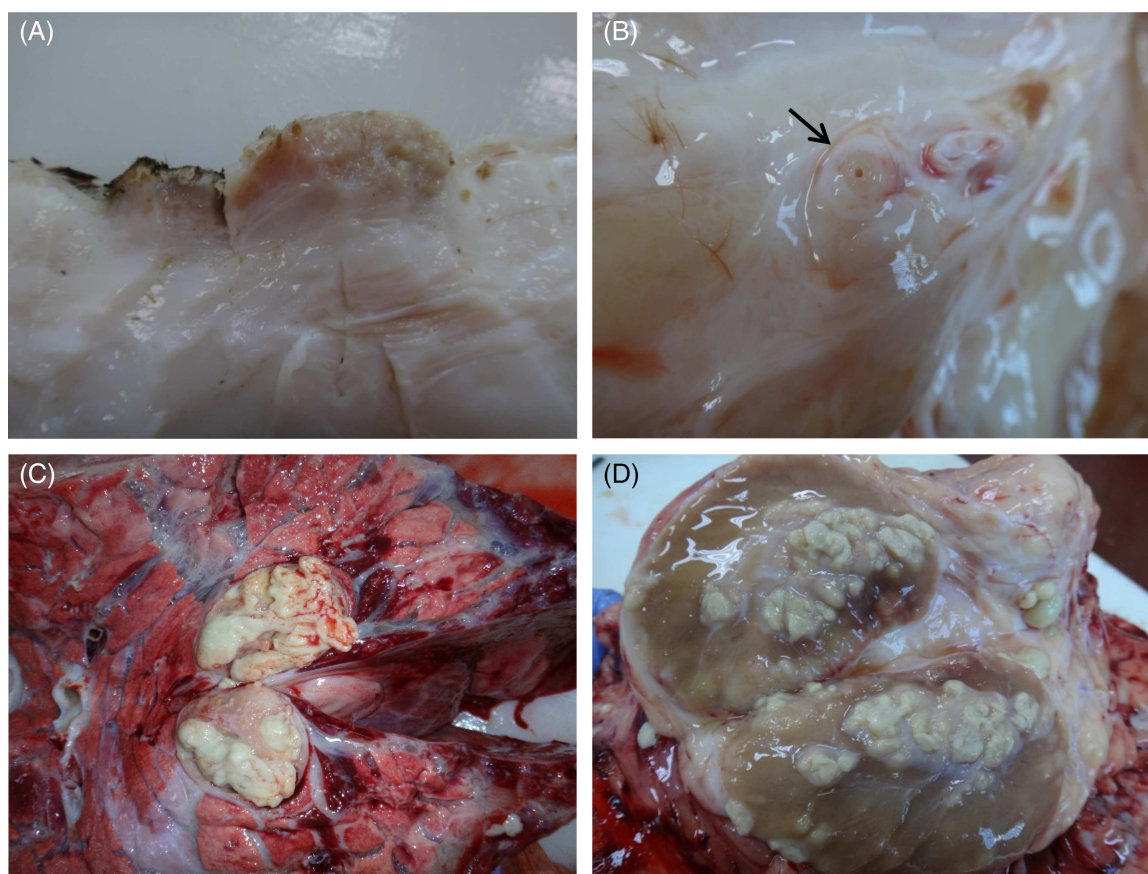


FIGURE 3 Main macroscopic lesions observed at necropsy of a 4-year-old Rouge-des-Prés cow (cow A) with actinobacillosis. A, Cutaneous nodule in the left hind limb; (B) lymphangitis in the left hind limb (pointed out by the arrow); (C) pulmonary thromboembolic abscess; (D) abscess in the left retromammary lymph node

for other clinical signs of actinobacillosis (particularly “wooden tongue”). The only potential risk factors were chorioptic mange and slightly abrasive straw used for bedding. No “wooden tongue” had been observed in this herd or in neighboring herds.

Cutaneous nodules on the udder of the third cow disappeared after administration of long-acting oxytetracycline (20 mg/kg, IM, q72h, 6 days). A new free-stall barn was built in summer 2015. Chorioptic mange reappeared in winter 2015–2016 despite topical treatment with ivermectin (500 µg/kg). One cow developed similar cutaneous lesions in its hind limb in February 2016. The animal recovered after administration of long-acting oxytetracycline (20 mg/kg, IM, q72h, 6 days).

4 | DISCUSSION

Actinobacillosis usually is considered a sporadic condition, typically involving the tongue in cattle (“wooden tongue”).⁶ Lymphadenitis and granulomatous skin lesions involving other tissues (oral cavity, fore-stomachs, skin of the head, neck and occasionally limbs) also have been reported,^{7–10} sometimes with several animals concomitantly affected in a single herd.^{9,11–13} In our report, 2 animals from a single herd had nodular dermatitis affecting the hind limbs with associated swelling, enlarged regional lymph nodes, pneumonia, and weight loss. One of the cows also had nodules in the udder skin and mastitis with purulent secretion. The clinical presentation, histopathological findings, and isolation of *A. lignieresii* from the different lesions confirmed the diagnosis of actinobacillosis. To our knowledge, ours is the first report of pneumonia caused by *A. lignieresii* associated with other lesions, in this case cutaneous actinobacillosis lesions that affected hind limbs in at least 2 cows from a single herd.

The failure of initial treatment and long course of the disease could explain the rather unusual clinical presentation of actinobacillosis in these cows. The udder parenchyma and lungs may have been affected secondary to the spread of *A. lignieresii* from cutaneous tissues through the lymphatics.⁶ Such a distribution presumably led to moderate to severe lymphangitis of the affected limb and subsequent lameness, which may have impaired the cow's ability to ingest food and subsequently led to progressive weight loss. Weight loss clearly was more severe in cow B, because this cow also had clinical paratuberculosis.

Cutaneous actinobacillosis has been reported to respond well to prolonged antimicrobial treatment, even when the lesions have been present for several months.³ In our cases, cutaneous lesions did not respond to the initial IM administration of long-acting oxytetracycline, despite the fact that the isolate was susceptible to tetracycline. Possibly, the treatment was too late or not long enough. Both cases that occurred later in the herd and were treated at the onset of cutaneous signs with long-acting oxytetracycline and repeated after 72 hours responded well. This observation emphasizes the need for prompt and sometimes prolonged antimicrobial treatment to successfully treat cutaneous actinobacillosis (ie, at least 6 days of treatment).

The first sign observed by the farmer in both cases was swelling in a hind limb. *Actinobacillus lignieresii* may have entered the cutaneous or SC tissues through a small puncture wound in the limb that was

contaminated by subsequent licking of the wound by the cow. Indeed, *A. lignieresii* is a normal inhabitant of the mouth and rumen of ruminants and is not believed to survive for more than 5 days in the environment.⁶ No risk factors for wounds were found by the referring veterinarian, except potentially abrasive straw used for bedding. Similar straw had been used in previous years, with no cows affected by cutaneous actinobacillosis. Wounds in the hind limbs also could have been facilitated by leg mange in this herd. No *Chorioptes* or other mites were found on the cows at referral. However, their occurrence could not be excluded because the cows had been treated with ivermectin and fenvalerate before referral. The difficulty that cows have licking their forelimbs could explain why, although forelimbs may have had wounds, only hindquarters were affected, as previously reported.^{11,12}

Identification of *A. lignieresii* from chronic lesions may be difficult, especially after antibiotic treatment.^{3,11} Histopathological examination of biopsy specimens thus usually is helpful for diagnosis. In our report, the typical histological lesion, that is, pyogranulomatous inflammation with Gram-negative organisms in the center bordered by eosinophilic club-like rosettes (or Splendore-Hoeppli phenomenon), was observed in all affected tissues, including the dermis, in both animals, as well as in the lungs, lymph nodes, and udder in cow A. A *Pasteurellaceae* organism was detected in all specimens submitted for bacteriological culture, but the phenotypic characterization was not sufficient to differentiate *A. lignieresii* from other bacteria such as *Bibersteinia trehalosi* or *Mannheimia haemolytica*. We thus used partial DNA gene sequencing to identify *A. lignieresii*.¹⁴ The development of a PCR test could be useful to more easily identify *A. lignieresii*.

In conclusion, our report emphasizes that *A. lignieresii* may affect tissues other than the tongue. Swelling in the hind limbs should promote careful examination of the skin for cutaneous or SC nodules and general clinical examination to detect any potential secondary infections, including pneumonia. Prompt and long-acting antimicrobial treatment should be implemented as soon as actinobacillosis is suspected by histopathological or microbiological analyses so as to avoid further dissemination and complications. Our report also suggests chorioptic mange as a predisposing factor. Mites should be sought, particularly when several animals are observed with cutaneous actinobacillosis.

ACKNOWLEDGMENTS

The authors thank the participating beef farmer and veterinarian. This case was presented as a poster at the 8th World Congress of Veterinary Dermatology (31 May to 4 June 2016, Bordeaux, France) and at the 29th World Buiatrics Congress (3 to 8 July 2016, Dublin, Ireland).

CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Authors declare no IACUC or other approval was needed.

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

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How to cite this article: Relun A, Cesbron N, Bourdeau P, et al. Atypical actinobacillosis affecting hind limbs and lungs in a single beef cattle herd. *J Vet Intern Med*. 2019;33:297-301. <https://doi.org/10.1111/jvim.15387>